PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Review

Cite this article: Young FJ, Montgomery SH. 2020 Pollen feeding in *Heliconius* butterflies: the singular evolution of an adaptive suite. *Proc. R. Soc. B* **287**: 20201304.
http://dx.doi.org/10.1098/rspb.2020.1304

Received: 5 June 2020
Accepted: 15 October 2020

Subject Category:
Behaviour

Subject Areas:
behaviour, cognition, evolution

Keywords:
behaviour, diet, brain size, life history, senescence, novelty

Authors for correspondence:
Fletcher J. Young
e-mail: fletcherjyoung@gmail.com
Stephen H. Montgomery
e-mail: s.montgomery@bristol.ac.uk

THE ROYAL SOCIETY
PUBLISHING

Pollen feeding in *Heliconius* butterflies: the singular evolution of an adaptive suite

Fletcher J. Young[1,2] and Stephen H. Montgomery[2]

[1]Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK
[2]School of Biological Science, University of Bristol, 24 Tyndall Avenue, Bristol UBS8 1TQ, UK

FJY, 0000-0002-2549-2107; SHM, 0000-0002-5474-5695

Major evolutionary transitions can be triggered by behavioural novelty, and are often associated with 'adaptive suites', which involve shifts in multiple co-adapted traits subject to complex interactions. *Heliconius* butterflies represent one such example, actively feeding on pollen, a behaviour unique among butterflies. Pollen feeding permits a prolonged reproductive lifespan, and co-occurs with a constellation of behavioural, neuroanatomical, life history, morphological and physiological traits that are absent in closely related, non-pollen-feeding genera. As a highly tractable system, supported by considerable ecological and genomic data, *Heliconius* are an excellent model for investigating how behavioural innovation can trigger a cascade of adaptive shifts in multiple diverse, but interrelated, traits. Here, we synthesize current knowledge of pollen feeding in *Heliconius*, and explore potential interactions between associated, putatively adaptive, traits. Currently, no physiological, morphological or molecular innovation has been explicitly linked to the origin of pollen feeding, and several hypothesized links between different aspects of *Heliconius* biology remain poorly tested. However, resolving these uncertainties will contribute to our understanding of how behavioural innovations evolve and subsequently alter the evolutionary trajectories of diverse traits impacting resource acquisition, life history, senescence and cognition.

1. Introduction: behaviour and the evolution of adaptive suites

Major evolutionary transitions can be triggered by behavioural novelty [1] and are often associated with 'adaptive suites' that incorporate multiple co-adapted traits [2]. These transitions involve complex interactions, including mutual dependency between traits [2]. In these circumstances, disentangling the causal relationships between behavioural and morphological traits involved in major transitions is challenging.

The butterfly genus *Heliconius* (Nymphalidae: Heliconiinae) presents an excellent system for investigating how adaptive suites evolve. Uniquely among butterflies, *Heliconius* supplement their nectar diet by actively collecting and feeding on pollen [3]. *Heliconius* gather pollen by probing flowers and collecting it as a lumped mass on the proboscis (figure 1*a*). The pollen load is mixed with saliva and externally digested to release amino acids that are drawn up the proboscis [3]. Pollen is collected primarily from a limited number of plant species [8], and *Heliconius* from at least the *melpomene* clade (figure 1*g*) show a particular preference for certain pollen-rich cucurbitaceous vines [8–10], with which they are hypothesized to have coevolved [11,12].

This dietary innovation provides adults with a consistent supply of amino acids, permitting a prolonged reproductive lifespan [13], and co-occurs with behavioural, neuroanatomical, life history, physiological and morphological changes that are absent in closely related, non-pollen-feeding Heliconiini [14] (figure 2). Among these putative adaptations, apparent specializations in foraging

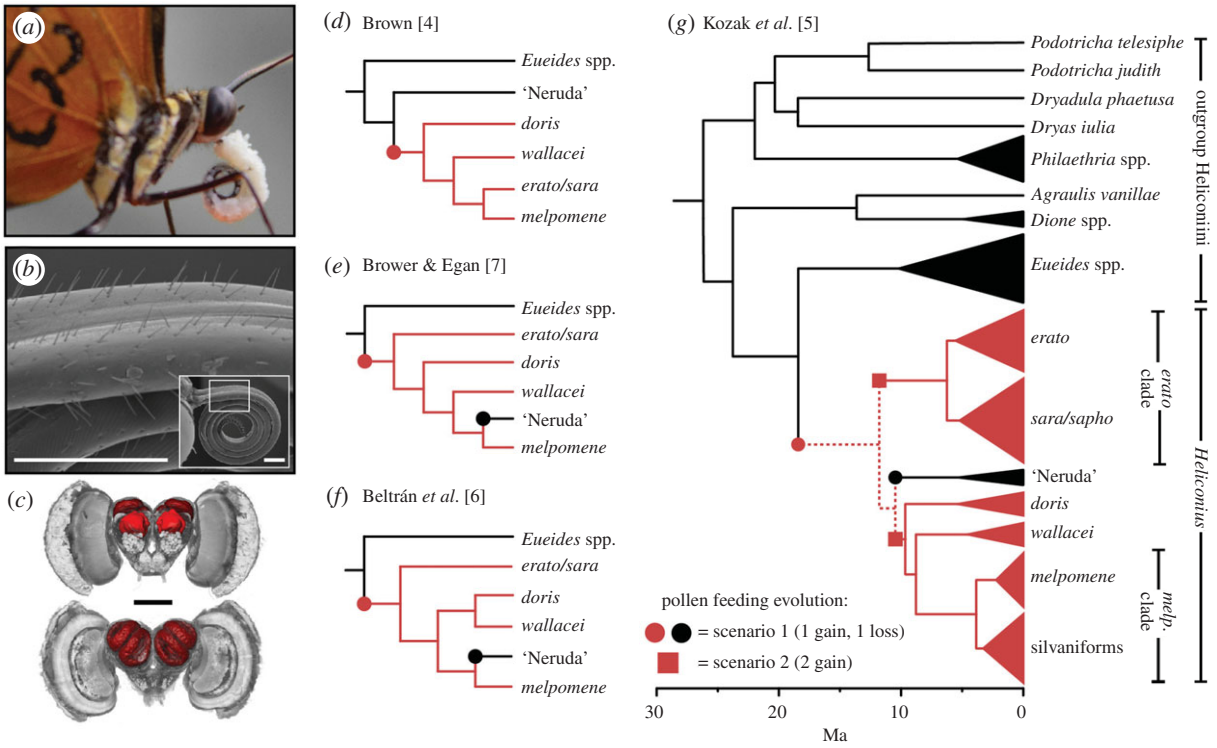

Figure 1. Evolution of pollen feeding. (*a*) A captive *H. hecale* with pollen load (white) affixed to its proboscis. (*b*) Elongated sensory bristles on the proboscis of *H. melpomene*. (*c*) A three-dimensional reconstruction of a *H. hecale* brain showing the mushroom bodies (red) and rest of the brain (grey) from the posterior (top) and anterior (bottom) view. Scale bars, 500 µm in (*b*) and (*c*). (*d–g*) How phylogenetic hypotheses for Heliconiini have changed through time, and how that affects predicted gains (red circle/square) and loss (black circle) of pollen feeding. Pollen feeding lineages are shown in red, dotted lines indicate branches with higher uncertainty. Note the changing position of the Neruda clade, a group of four non-pollen feeding species. (*d*) Adapted from [4] and based on morphological data. (*e–g*) Adapted from [5–7] and based on molecular data. (Online version in colour.)

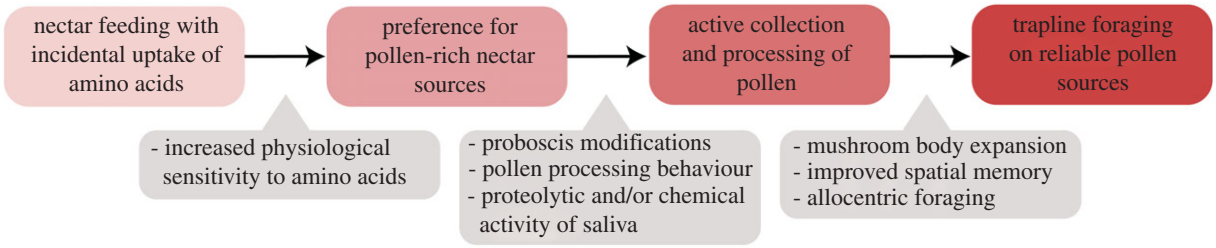

Figure 2. Hypothesized transition for a butterfly from nectarivory to trapline pollen feeding, showing plausible intermediary stages and possible key adaptations. (Online version in colour.)

behaviour have received particular attention. *Heliconius* establish 'traplines', foraging routes along which specific plants are regularly visited, suggesting a sophisticated capacity for spatial navigation, probably using learnt visual landmarks [11,15,16].

Pollen feeding evolved relatively recently, with *Heliconius* and its sister genus *Eueides* diverging approximately 18 Ma [5]. The close phylogenetic relatedness and ecological similarity with other Heliconiini therefore provides a potentially powerful framework for conducting comparative analyses. However, the suite of traits associated with pollen feeding is widely assumed to have evolved only once [6] (figure 1*d–g*), presenting difficulties in separating evolutionary cause and effect. As such, many hypothesized links between different aspects of *Heliconius* biology are poorly tested. Moreover, no physiological, morphological or molecular traits have been specifically linked to the origin of pollen feeding in this genus. Nevertheless, as a major system for studying the genomic bases of adaptation [14], considerable genomic resources have been developed for *Heliconius* [17]. Together with recently developed genetic techniques and comparative methods [18–20], this provides a clear route towards understanding the evolution of this behavioural innovation.

This review synthesizes current knowledge of pollen feeding in *Heliconius*, highlighting gaps in our understanding of several putatively linked traits, and their interactions, which are probably key to the origin of pollen feeding. We identify key areas of investigation that can contribute to central questions in evolutionary biology including: (i) how dietary shifts can alter energic constraints, re-shaping life history and reproductive trade-offs; (ii) how evolutionary innovations co-opt ancestral molecular and morphological traits; and (iii) how brains accommodate cognitive enhancements.

2. Pollen-feeding in *Heliconius* butterflies: an energetic payload

Populations are often under energetic constraint, resulting in investment trade-offs between competing tissues, traits, or strategies [21]. Shifts in diet quality have dramatic effects on

evolutionary trajectories by increasing individual energy budgets. For example, dietary innovations have been linked to larger body sizes in carnivores [22], brain expansion in primates [23] and increased fecundity in butterflies [24]. However, comparative life-history studies often compare phylogenetically and ecologically diverse species, leading to difficulties in determining the precise role of dietary shifts in macro-evolutionary dynamics (see [25]).

Like most holometabolous insects, Lepidoptera experience a profound shift in nutrient intake between the larval and adult stages. Lepidopteran larvae are generally herbivorous, whereas adults of most species feed predominantly on nectar, which is carbohydrate-rich, but protein-poor [26,27]. Consequently, reproductive output is largely constrained by protein acquired during the larval stage [24,26]. While some Lepidoptera also exploit alternative nutrient sources including mud, fruit, dung and carrion [27], pollen feeding in *Heliconius* is perhaps the clearest example of a change in adult diet being linked to major shifts in life history. Pollen feeding provides *Heliconius* with a consistent source of essential amino acids during the adult stage [3]. Compared to other Heliconiini, *Heliconius* exhibit a pronounced delay in female reproductive senescence [13]. *Heliconius* females generally collect more pollen than males [9,28], incorporating pollen-derived amino acids into eggs [29], and suffer a marked decline in fecundity when deprived of pollen [13]. This reflects a potential physiological convergence with honeybees, where colonies cease brood rearing when denied access to pollen [30].

Heliconius butterflies have dramatically extended lifespans, living for up to six months in the wild, without diapause [3,15], compared to a maximum of four to six weeks in *Dryas iulia*, a non-pollen-feeding Heliconiini [13]. However, the causal relationship between pollen feeding and longevity is not well quantified. One study reports that pollen-deprived *Heliconius charithonia* are shorter-lived [13], but this difference was not tested statistically. In adult honeybees, pollen feeding is associated with increased longevity [31], providing reason to expect a similar effect in *Heliconius*.

The energetic payload provided by pollen feeding has clearly had a major impact on reproductive output in *Heliconius*. This provides several opportunities for investigating the effects of foraging innovations on life histories, for example: (i) how do novel diets alter energetic trade-offs between life stages? (ii) what physiological mechanisms control the shifting balance of these trade-offs and the use of new resources? and (iii) what physiological mechanisms underpin increased longevity?

3. Origins of a novel trait

The processes and underlying conditions that give rise to evolutionary novelties are incompletely understood [32]. Although evolutionary novelties can arise from the emergence of new ecological opportunities [33], this is not always the case [34]. Importantly, evolutionary trajectories appear constrained by pre-existing variation [35,36], suggesting contingency plays a substantial role in the emergence of novel traits. For example, complex behaviours can be achieved through the integration of simpler, pre-existing behavioural modules [37]. However, the relative importance of behaviour, morphology and physiology as the ultimate drivers of novelty is debated [32]. Understanding fitness benefits during intermediate stages, and the timing of trait acquisition, is therefore key to disentangling the origins of an innovation.

(a) Reconstructing evolutionary shifts in pollen feeding

Except for the four species of the 'Neruda' clade (figure 1*e–g*), all *Heliconius* species feed on pollen and appear to possess the complete suite of associated traits, presenting a challenge to reconstructing the origin of pollen feeding. As the only non-pollen-feeding *Heliconius*, the four Neruda species may offer the possibility of decoupling pollen feeding and its associated adaptations, helping to resolve the timing of these shifts, and the relationships between traits. Regrettably, such analyses are currently limited by the scarcity of data on Neruda biology and lingering uncertainty regarding their phylogenetic position. Long considered a separate genus [4] (figure 1*d*), recent molecular phylogenies have positioned Neruda within *Heliconius* [5–7] (figure 1*e–g*). Hence, whether pollen feeding evolved once in *Heliconius* and was secondarily lost in the Neruda, or evolved multiple times within *Heliconius*, with Neruda retaining the ancestral state, is unclear, with these two scenarios being equally parsimonious (figure 1*g*) [5]. In addition, given evidence of widespread introgression throughout the evolution of the genus [17], which could mislead the species tree, it also remains possible that the Neruda are, after all, a sister clade to *Heliconius*, as suggested by morphological data (figure 1*d*). Nevertheless, the absence of pollen feeding in other Heliconiini suggests that a single gain within *Heliconius* is likely, with or without a loss in the Neruda. However, discordance between any single Heliconiini species tree and underlying gene trees may present persistent difficulties in resolving this question [38].

More broadly, the scarcity of pollen feeding across the approximately 180 000 described species of Lepidoptera marks *Heliconius* as particularly peculiar. Although wild-caught butterflies from several genera have been reported as having pollen affixed to their proboscis [39], *active* pollen feeding is unknown in other Lepidoptera, with only a few exceptions, all of which are separated from *Heliconius* by large phylogenetic distances. Two families of basal moths, Heterobathmiidae and Micropterigidae, feed on pollen as adults [40]. These groups, however, lack a proboscis and use plesiomorphic mandibles to collect and crush pollen [40]. Two species of Gelechiidae moths are the only other proboscis-bearing Lepidoptera reported to feed on pollen, purportedly by dissolving the pollen wall with an unidentified salivary agent [41]. However, the ecology and life history of these species, and the prevalence of pollen feeding across Gelechiidae, are poorly understood, making it difficult to assess the feasibility of comparative analyses within this group.

(b) Fitness during evolutionary transitions: effects of adult amino acid intake

The presence of amino acids in nectar [42], often derived from contaminating pollen grains [43], suggests that pollen feeding may have originated with the incidental intake of amino acids while nectar feeding. This may have selected for an increased sensitivity to amino acids, and a preference for pollen-rich plants (figure 2). The importance of nectar-derived amino acids to adult butterflies is supported by comparisons showing that plants pollinated by butterfly species tend to have

higher amino acid concentrations in their nectar [42]. Additionally, several butterfly species show a preference for nectars containing amino acids [44,45]. However, the benefits derived from amino acids in the adult diet vary widely between Lepidopteran species. In several species, no association has been found between fecundity and adult amino acid intake [46–48]. Yet, for other species, it has been linked to increases in egg quantity [49] and size [50]. Importantly, the transfer of nectar-derived amino acids to eggs has been directly demonstrated in some species [51,52]. The effects on male fecundity have received less attention, however, male *Coenonympha pamphilus* produced larvae with larger hatching masses when provided with amino acids, probably owing to enhanced spermatophore quality [53]. Adult amino acid intake also increases longevity in some species, but has no effect in others [46,54]. Interestingly, lifespans comparable to *Heliconius* have been recorded in some fruit-feeding butterflies [55], raising the possibility that fruit-derived amino acids may similarly facilitate an extended lifespan.

While the effects appear to be highly species-dependent, evidence that adult amino acid intake can lead to improvements in longevity or fecundity supports the hypothesis that pollen feeding in *Heliconius* probably originated from passive uptake of pollen-derived amino acids during nectar feeding (figure 2). If so, it remains puzzling that pollen feeding is so rare. Central to answering why, is identifying the adaptations necessary for the transition to pollen feeding.

(c) Using old traits for new purposes

Comparative studies across Heliconiini suggest that pollen collection does not involve any novel morphological structures [56]. However, *Heliconius* do have elongated proboscises compared to non-pollen-feeding Heliconiini, with longer and more numerous bristles at the proximal- and mid-regions (figure 1*b*), which may assist in affixing pollen grains [56]. In addition, the intrinsic muscles involved in coiling movements are more numerous and extend further into the proboscis [57]. Pollen collection also involves the same sequence of probing movements as nectarivorous butterflies [27,58], but *Heliconius* probe with higher frequency and handle individual flowers for longer, with handling time increasing in the presence of pollen [27,58]. Additionally, pollen processing may be derived from proboscis grooming behaviours, which similarly involve the release of saliva and the repetitive coiling of the proboscis [59].

Saliva appears to play a key role in pollen feeding by helping to bind pollen to the proboscis and facilitating external digestion. Indeed, the salivary glands of *Heliconius* are larger than in other nymphalids [60], and *Heliconius* release greater quantities of saliva during feeding [58]. Although the saliva of *Heliconius* contains proteases [61,62], it is unknown how it differs from that of other Heliconiini. However, proteolytic activity of the saliva does increase when the proboscis is stimulated with pollen, and is generally higher in females [61]. Two of the proteases identified in *Heliconius melpomene* saliva also show close homology with the serine protease *cocoonase* [62,63], which is secreted from the proboscis of silkworms to weaken the cocoon during eclosion [64]. Like all butterflies, *Heliconius* lack cocoons, and it has been suggested that *cocoonase* homologues may have been co-opted for use in digesting pollen proteins [62,63], potentially weakening the pollen wall. *Cocoonase* underwent several duplications along the lineage leading to *Heliconius* and their sister genus, *Eueides*, with an additional duplication specific to *Heliconius* and a further duplication in *H. melpomene* [63]. However, a functional role for *cocoonase* in pollen feeding has not yet been directly demonstrated. Importantly, it is unclear how proteolysis would break down sporopollenin, the primary component of pollen exines, as it is not composed of proteins [65]. Pollen grains are, in fact, visibly damaged after processing by *Heliconius* [66]. However, it is unknown if this is achieved solely by mechanical digestion, or through biochemicals capable of breaking down sporopollenin, as is claimed for Gelechiidae moths [41]. The role of specific salivary proteins in pollen digestion therefore requires further comparative studies that include a broader representation of the non-pollen-feeding Heliconiini.

Pollen feeding illustrates how evolutionary innovation can occur through the co-option and modification of pre-existing anatomical, behavioural and physiological traits for new purposes. Although it remains unclear why pollen feeding arose in *Heliconius* but not other butterflies, this question can potentially be answered by combining functional genetics, physiology and anatomy.

4. Increases in behavioural sophistication and neural elaboration

Dietary innovations not only involve adaptations in the processing and use of a resource but often co-occur with changes in foraging behaviour as determined by the quality, and spatial and temporal distribution of food sources [67]. These parameters impose demands on perception, learning and memory, and can favour investment in associated brain structures [68]. In *Heliconius*, the interactions between butterflies and their pollen sources may have led to notable refinements in both the brain and behaviour [11,16,69,70].

(a) Exploitation of a novel resource: plant–animal interactions and foraging strategies

Many species of *Heliconius* collect pollen predominantly from cucurbitaceous vines, particularly the relatively rare, but pollen rich *Psiguria* and *Gurania*, which show evidence of co-evolution with *Heliconius* [8–12]. *Heliconius* are the primary pollinators of *Psiguria*, visiting more plants, and depositing more pollen, over greater distances, than even hummingbirds [71]. Indeed, several species of *Gurania* and *Psiguria* appear to have evolved lower nectar production and smaller flowers to promote visitation from *Heliconius* over hummingbirds, and older plants may even switch from producing male to female flowers once *Heliconius* visitation is established [72].

Psiguria flowers contain large amounts of pollen, and inflorescences generally produce a new flower every 1–3 days [11]. Individual plants can flower continuously for up to a year [11], in contrast to the seasonal pollen production common for neotropical angiosperms [73]. A single *Psiguria* plant is therefore potentially a reliable pollen resource for the entire lifespan of an individual. *Heliconius* use this dependable, but scarce, resource by establishing 'traplines', foraging routes along which specific plants are visited with a high degree of spatial and temporal regularity [11,15,16]. This suggests *Heliconius* possess a capacity for navigation using learnt visual landmarks, similar to behaviours observed in certain

bees [74,75]. *Heliconius* traplines centre on a limited home range of 100 m^2 to 1 km^2, within which individuals return to the same roosting locations at night, located using visual cues [16,71,76]. Although other butterfly species, including the heliconiine *Agraulis vanillae*, are reported to temporarily establish home ranges [77], *Heliconius* seem peculiar in maintaining long-term, stable home ranges with high roost-site fidelity [16,71]. Site fidelity is presumably a pre-requisite for trap lining, which, together with the central role of pollen resources in *Heliconius* trap lines, suggests that these behaviours are linked to the acquisition of pollen feeding. However, the lack of data on whether non-pollen feeding Heliconiini use spatial information during foraging, means this hypothesis is yet to be formally tested.

Despite the role of *Psiguria* and *Gurania* in *Heliconius* foraging behaviour, there is considerable variation in the exploitation of pollen sources between species. Although these differences are partly explained by habitat preference, there appears to be a division between the two main *Heliconius* clades [8]. Species of the *melpomene* clade tend to forage more intensively on *Psiguria*, while *erato* clade species predominantly exploit non-cucurbitaceous plants, such as *Lantana* [8–10] (figure 1*g*). Notably, *Heliconius erato* do trapline on specific patches of *Lantana* [16]. Hence, the role of cucurbitaceous pollen sources in the transition to pollen feeding is unclear. One possibility is that pollen feeding arose in the context of coevolution with certain cucurbitaceous vines, with members of the *erato* clade subsequently pushed towards other pollen resources owing to competitive exclusion. Alternatively, pollen feeding may have originated as a more opportunistic, generalist strategy, retained in the *erato* clade, with specialization on Cucurbitaceae secondarily emerging in the *melpomene* group.

(b) Neural basis of a cognitive adaptation

Behavioural innovations are generally associated with changes in the structure and function of the brain [78]. For example, foraging innovations are linked to brain expansion in several vertebrate groups [23,79]. Trapline foraging in *Heliconius* represents a degree of behavioural sophistication rarely reported among the Lepidoptera, and is suggestive of enhancements in visually oriented spatial memory and long-term memory retention [11]. The cognitive abilities of *Heliconius* relative to non-pollen-feeding Heliconiini are, however, yet to be experimentally assessed. Nevertheless, the apparent cognitive demands of traplining are predicted to be associated with elaborations in the *Heliconius* nervous system [69,70].

Indeed, *Heliconius* have dramatically enlarged mushroom bodies (figure 1*c*), which are three to four times larger than is typical of Lepidoptera, including two closely related Heliconiini, *Dryas iulia* and *Agraulis vanillae* [69,70]. Mushroom bodies are paired, central brain structures that receive visual and/or olfactory information, and play an important role in learning and memory [80]. Mushroom body function varies across insects with different ecologies, which is reflected in the relative importance of visual and olfactory inputs [80]. In *Heliconius*, the demands of trapline foraging is hypothesized to have driven mushroom body expansion [69,70]. However, direct evidence for a functional link between the mushroom bodies and visually oriented spatial memory is limited to a handful of ablation experiments in cockroaches [81] and ants [82,83]. Indirect evidence also comes from comparative data from Hymenoptera, showing that expansion of the mushroom bodies coincided with the evolution of parasitoidism [84], which relies on spatial memory for host location [85], and plasticity experiments in a desert ant that show visually guided foraging experience affects mushroom body maturation [86]. Though suggestive, these data are relatively impoverished compared to our understanding of the role of the central complex, another sensory-motor integration structure in the central brain, in insect spatial learning and orientation [87,88].

Increases in *Heliconius* mushroom body size have also been speculatively linked to host plant use [89]. *Heliconius* lay exclusively on *Passiflora* plants, with varying degrees of specialization. *Passiflora* display a remarkable diversity of leaf shape, and host plant use in *Heliconius* appears to be, in part, based on leaf shape recognition and learning through associative conditioning [90]. Mushroom body expansion may, therefore, support a greater array of search images and enhanced shape-learning abilities, facilitating improved visual identification of host plants [89]. Indeed, there are indications that, for some butterflies, mushroom body plasticity is shaped by experience with host plants [91,92]. However, the current lack of data on variation in mushroom body size within *Heliconius* and across Heliconiini prohibits tests of these hypotheses. Likewise, a better understanding of the foraging behaviours of non-pollen-feeding Heliconiini is crucial to understanding the drivers behind mushroom body expansion in *Heliconius*.

The impacts of pollen feeding in *Heliconius* clearly extend beyond direct effects on fecundity, shaping their foraging strategy and probably changing the types and complexity of information processed and stored by the brain. *Heliconius* therefore offer a highly tractable system for investigating how behavioural innovations can alter a species' cognitive ecology.

5. Ripple effects following evolutionary innovations

Profound shifts in a major trait can have substantial knock-on effects on the selection regimes governing both developmental and evolutionary processes [2] (figure 3). In *Heliconius*, the evolution of pollen feeding has been linked to an extensive suite of life history, reproductive and phenotypic traits. However, the interactions and dependencies between these traits are poorly explored. Here, we highlight three areas in which pollen feeding is hypothesized to have altered *Heliconius* biology (figure 3), and which illustrate the broader impacts of behavioural innovations.

First, dietary improvements in adults can alter how energetically expensive investments are provisioned during the larval stage [28] (figure 3, green). As larvae, Heliconiini sequester cyanogenic compounds and synthesize cyanogens de novo from host plant (*Passiflora*) derived amino acids [99], making them distasteful to predators. A pollen-rich adult diet may allow *Heliconius* to allocate a greater proportion of larval resources towards chemical defence, rather than energy reserves, with the shortfall in reproductive investment being recouped during the adult stage [100]. However, although *Heliconius* tend to emerge with higher cyanogen concentrations than most other Heliconiini, the non-pollen-feeding *Agraulis vanillae* and *Eueides* spp. show levels similar to *Heliconius* [100,101]. Such comparisons are, however, complicated by adult cyanogen profiles being influenced by larval diet

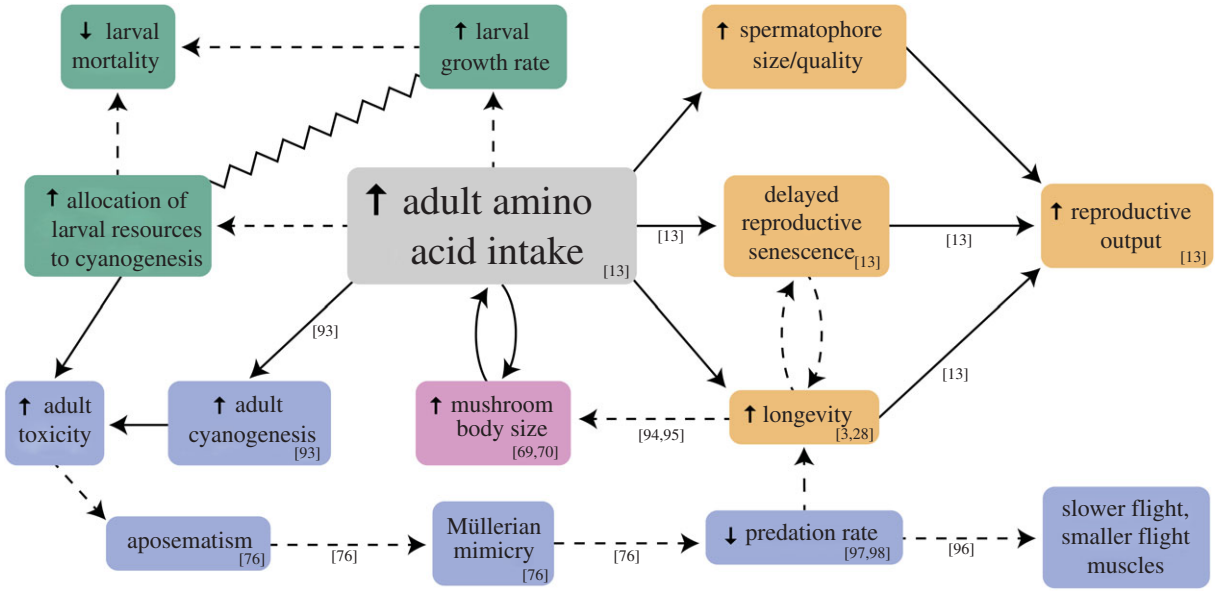

Figure 3. Summary of the hypothesized consequences of increased adult amino acid intake in *Heliconius*, divided into: (i) changes in the allocation of larval resources, green; (ii) increased longevity and delayed reproductive senescence, yellow; (iii) increases in cyanogenesis, adult distastefulness and aposematism, blue; and (iv) increases in behavioural sophistication and neural elaboration, pink. Solid arrows represent amino acid investment, dashed arrows represent selective pressures, and the zig-zag line represents a developmental trade-off. Footnotes indicate supporting evidence for specific traits or relationships. (Online version in colour.)

[99,102], which varies between and within Heliconiini species. Moreover, cyanogen concentration is an imperfect proxy for distastefulness due to interspecific variation in chemical profile [101]. Pollen feeding has also been suggested to allow *Heliconius* to reduce time spent in the vulnerable larval stage [3,12] by trading off increased growth rates against investment in nitrogen reserves. Although larval growth rates across the Heliconiini have not yet been compared, females *Dryas iulia* emerge with a higher proportion of abdominal nitrogen allocated to reproductive reserves than *Heliconius* [28]. Similarly, compared with *D. iulia*, *H. charithonia* females emerge with smaller ovaries containing fewer oocytes, further suggesting a reduction in larval investment in reproduction [13].

Second, it has been suggested that *Heliconius* may also appropriate pollen-derived amino acids for increased cyanogenesis during the adult stage [12]. Together with a potential increased allocation of larval resources to chemical defence, this could strengthen selection for aposematic wing patterns and Müllerian mimicry [76] (figure 3, blue). *Heliconius* do experience lower predation rates than sympatric non-pollen-feeding Heliconiini [97,98], indicating greater unpalatability and salience of warning cues, which, in butterflies, is also associated with decreased investment in flight muscle [96]. However, the role of pollen intake in adult cyanogenesis remains ambiguous. Restriction of dietary amino acids reportedly has no effect on cyanogen levels in two-week old *Heliconius ethilla* and *Heliconius hecale* [100]. However, an effect was seen in *H. melpomene* after two to four weeks, suggesting pollen feeding could *prolong* adult cyanogenesis [93]. *Heliconius* also invest large amounts of cyanogens in eggs and spermatophores [99], and pollen feeding may help replenish cyanogens lost through reproduction. Indeed, when deprived of amino acids, *H. charithonia* show depressed cyanogen levels relative to freshly eclosed adults, suggesting that cyanogens are re-appropriated under stressful conditions [100].

Finally, a decrease in adult mortality owing to predation, together with a delay of reproductive senescence, probably strengthened selection for extended lifespans (figure 3, yellow). An increase in longevity would also favour investment in learning, long-term memory and their neural correlates (figure 3, pink), which is less rewarding for shorter-lived species [94,95]. However, this interaction may itself require concomitant investment trade-offs or physiological adaptations, as the costs of learning can cause *reductions* in longevity [103], and fecundity [104]. While the above hypotheses remain poorly tested, interactions between these diverse traits may be crucial to the evolution of pollen feeding [25].

6. Disentangling the origins of a singular adaptation

Since it was first described nearly 50 years ago [3], pollen feeding in *Heliconius* has been shown to involve a complex suite of adaptations (figure 2), with substantial knock-on effects on life history (figure 3). We propose one evolutionary scenario that describes plausible transitional stages in moving from nectarivory to trapline pollen feeding (figures 2 and 3), highlighting important ecological and evolutionary interactions. However, the order in which these traits were acquired, and therefore how they interact, is unresolved. Nevertheless, the comparatively recent phylogenetic scale over which these shifts occurred (approx. 18 Ma [5]) positions *Heliconius* as a valuable model for exploring a number of core questions in evolutionary biology. Importantly, *Heliconius* are already a highly tractable system, readily reared in insectaries and amenable to behavioural experimentation [90], and supported by broad genomic resources [17] and tools for studying candidate genes [18,20]. Below, we identify five fundamental questions in evolutionary biology that can be addressed through further investigation of pollen feeding in *Heliconius*.

(a) How quickly do behavioural innovations alter selection on related traits?

Heliconius are distinguished from their non-pollen feeding relatives by several traits. However, whether these traits evolved in a short burst of multi-trait adaptive evolution, or were assembled gradually through time, is unclear. Variation

in the degree of specialization on cucurbit vines across *Heliconius* may provide one avenue to investigate ties between pollen feeding and foraging behaviours. However, as the only non-pollen-feeding *Heliconius*, the four Neruda species offer the possibility of separating pollen feeding from its associated adaptations. Although little is known about Neruda biology, particularly their foraging behaviour, there is evidence that they exhibit some ancestral traits. For example, the proboscis of *Heliconius aeode* lacks the increased density and length of bristles seen in other *Heliconius* [56]. However, the extent to which they share other traits with non-*Heliconius* Heliconiini is unclear. Unresolved questions include whether the Neruda trapline forage or possess expanded mushroom bodies, and the level of proteolytic activity in their saliva.

(b) What molecular mechanisms underlie functional innovations?

The nature of the molecular bases of adaptation is a central question in evolutionary biology, and identifying the genetic basis of a trait can help understand adaptive novelty [20]. For *Heliconius*, it is possible that developing the molecular 'toolkit' to digest pollen was a key innovation, unlocking a new ecological resource that triggered subsequent adaptive evolution in other traits. Although a number of proteases have been identified in *Heliconius* saliva [61,62], their role in pollen digestion is undemonstrated, and the mechanisms by which *Heliconius* saliva can break down pollen remain unclear. Comparisons of salivary compounds and gene expression in the mouthparts of *Heliconius* and other Heliconiini may lead to the identification of genes key to the evolution of pollen feeding, offering important insight into how novelty evolves.

(c) How do biological trade-offs shape the evolution of novel traits?

Novel traits often involve considerable costs and may only confer fitness benefits under certain conditions, resulting in unequal landscapes of adaptive opportunity between species [25,32]. Despite pollen feeding offering large reproductive benefits, *Heliconius* are the only butterflies to have evolved this ability. It is possible that reliable collection of pollen can only be achieved through increased investment in neural tissue and learning, both of which can be costly [105,106]. The benefits of increased reproductive longevity may also depend on high adult survival, which is supported by Müllerian mimicry in *Heliconius*. These interactions could be approached using mathematical models that formalize their interdependencies [107], or agent-based simulations that reveal a hierarchy of competitive advantages provided by different traits [108]. This provides one route to reconstructing the order in which these traits changed, and their inter-dependencies, highlighting which were key to the origins of pollen-feeding and which were consequences of it.

(d) What selective pressures and constraints shape brain evolution?

Expansion of specific brain regions is restricted by the energetic costs of neural tissue [106] and constraints that can limit the independent evolution of component parts [109]. Yet, region-specific changes can underpin behavioural innovation [109]. In *Heliconius*, increased behavioural sophistication putatively co-occurs with an expansion of the mushroom bodies. This provides an opportunity to study how selection for behavioural innovation can shape brain evolution, and how ancestral neural structures can be co-opted for new functions. Difficulties associated with testing comparative hypotheses where phenotypic shifts occur in a single lineage [110] may be overcome with more flexible phylogenetic methods that incorporate rate heterogeneity in trait evolution to reveal distinct shifts in correlated traits across time [19].

(e) What mechanisms permit delayed reproductive senescence and extended lifespans?

Age-related declines in fitness are widespread among animals, but these effects can vary dramatically between closely related taxa and within species [111]. However, our understanding of these processes remains incomplete. The greatly extended lifespans of *Heliconius* indicate a remarkable ability to delay bodily senescence [13]. Additionally, the ability to maintain long-term memories of foraging routes suggest that *Heliconius* may mitigate cognitive senescence observed in other insects [112,113]. Uncovering the mechanisms by which *Heliconius* delay senescence, and the potential role of pollen-derived amino acids, could provide valuable insight into ageing processes.

7. Conclusion

In conclusion, we highlight pollen feeding in *Heliconius* as a remarkable example of a behavioural innovation triggering an adaptive shift across a suite of multiple, interrelated traits, with the potential to become a highly informative, textbook case of the causes and consequences of behavioural evolution. By exploring the questions set out above, we can make progress towards better understanding how behavioural novelties arise, and subsequently lead to profound changes across diverse aspects of an animal's biology.

Data accessibility. This article has no additional data.

Authors' contributions. F.J.Y. and S.H.M. conceived of and wrote the manuscript.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by a Trinity College (Cambridge) Studentship to F.J.Y., a NERC IRF grant no. (NE/N014936/1) and an ERC Starter grant no. (758508) to S.H.M.

Acknowledgements. We thank the Montgomery and McMillan laboratories, Richard Merrill, Chi-Yun Kuo, Érika Pinheiro de Castro and Amar Sarkar for discussion and comments on the manuscript, Wyatt Toure for the image in figure 1*a*, and Harald Krenn for the image in figure 1*b*.

References

1. Zuk M, Bastiaans E, Langkilde T, Swanger E. 2014 The role of behaviour in the establishment of novel traits. *Anim. Behav.* **92**, 333–344. (doi:10.1016/j.anbehav.2014.02.032)

2. Pianka ER. 1981 Resource acquisition and allocation among animals. In *Physiological ecology: an*

evolutionary approach to resource use (eds P Calow, CR Townsend), pp. 300–314. Sunderland, MA: Sinauer.

3. Gilbert LE. 1972 Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proc. Natl Acad. Sci. USA* **69**, 1403–1407. (doi:10.1073/pnas.69.6.1403)
4. Brown KS. 1981 The biology of *Heliconius* and related genera. *Annu. Rev. Entomol.* **26**, 427–457. (doi:10.1146/annurev.en.26.010181.002235)
5. Kozak KM, Wahlberg N, Neild AFE, Dasmahapatra KK, Mallet J, Jiggins CD. 2015 Multilocus species trees show the recent adaptive radiation of the mimetic *Heliconius* butterflies. *Syst. Biol.* **64**, 505–524. (doi:10.1093/sysbio/syv007)
6. Beltrán M, Jiggins CD, Brower AVZ, Bermingham E, Mallet J. 2007 Do pollen feeding, pupal-mating and larval gregariousness have a single origin in *Heliconius* butterflies? Inferences from multilocus DNA sequence data. *Biol. J. Linn. Soc.* **92**, 221–239. (doi:10.1111/j.1095-8312.2007.00830.x)
7. Brower AVZ, Egan MG. 1997 Cladistic analysis of *Heliconius* butterflies and relatives (Nymphalidae: Heliconiiti): a revised phylogenetic position for Eueides based on sequences from mtDNA and a nuclear gene. *Proc. R. Soc. Lond. B* **264**, 969–977. (doi:10.1098/rspb.1997.0134)
8. Estrada C, Jiggins CD. 2002 Patterns of pollen feeding and habitat preference among *Heliconius* species. *Ecol. Entomol.* **27**, 448–456. (doi:10.1046/j.1365-2311.2002.00434.x)
9. Cardoso MZ. 2001 Patterns of pollen collection and flower visitation by *Heliconius* butterflies in southeastern Mexico. *J. Trop. Ecol.* **17**, 763–768. (doi:10.1017/S0266467401001572)
10. Boggs CL, Smiley JT, Gilbert LE. 1981 Patterns of pollen exploitation by *Heliconius* butterflies. *Oecologia* **48**, 284–289. (doi:10.1007/BF00347978)
11. Gilbert LE. 1975 Ecological consequences of a coevolved mutualism between butterflies and plants. In *Coevolution of animals and plants* (eds LE Gilbert, PH Raven), pp. 210–240. Austin, TX: University of Texas Press.
12. Gilbert LE. 1991 Biodiversity of a Central American *Heliconius* community: pattern, process, and problems. In *Plant–animal interactions: evolutionary ecology in tropical and temperate regions* (eds PW Price, TM Lewinsohn, GW Fernandes, WW Benson), pp. 403–427. New York, NY: Wiley.
13. Dunlap-Pianka H, Boggs CL, Gilbert LE. 1977 Ovarian dynamics in Heliconiine butterflies: programmed senescence versus eternal youth. *Science* **197**, 487–490. (doi:10.1126/science.197.4302.487)
14. Jiggins CD. 2017 *The ecology and evolution of* Heliconius *butterflies*, 1st edn. Oxford, UK: Oxford University Press.
15. Ehrlich PR, Gilbert LE. 1973 Population structure and dynamics of the tropical butterfly, *Heliconius ethilla*. *Biotropica* **5**, 69–83. (doi:10.2307/2989656)
16. Mallet J. 1986 Gregarious roosting and home range in *Heliconius* butterflies. *Natl Geogr. Res.* **2**, 198–215. (doi:10.2307/5054)
17. Edelman NB *et al.* 2019 Genomic architecture and introgression shape a butterfly radiation. *Science* **366**, 594–599. (doi:10.1126/science.aaw2090)
18. Lewis JJ, van der Burg KRL, Mazo-Vargas A, Reed RD. 2016 ChIP-seq-annotated *Heliconius erato* genome highlights patterns of *cis*-regulatory evolution in Lepidoptera. *Cell Rep.* **16**, 2855–2863. (doi:10.1016/j.celrep.2016.08.042)
19. Nunn CL, Zhu L. 2014 Phylogenetic prediction to identify 'evolutionary singularities'. In *Modern phylogenetic comparative methods and their application in evolutionary biology: concepts and practice* (ed. LZ Garamszegi), pp. 481–514. Berlin, Germany: Springer.
20. Concha C *et al.* 2019 Interplay between developmental flexibility and determinism in the evolution of mimetic *Heliconius* wing patterns. *Curr. Biol.* **29**, 3996–4009; e4. (doi:10.1016/j.cub.2019.10.010)
21. Stearns S. 1989 Trade-offs in life-history evolution. *Br. Ecol. Soc.* **3**, 259–268. (doi:10.2307/2389364)
22. Carbone C, Mace GM, Roberts SC, Macdonald DW. 1999 Energetic constraints on the diet of terrestrial carnivores. *Nature* **402**, 286–288. (doi:10.1038/46266)
23. DeCasien AR, Williams SA, Higham JP. 2017 Primate brain size is predicted by diet but not sociality. *Nat. Ecol. Evol.* **1**, 1–7. (doi:10.1038/s41559-017-0112)
24. Swanson EM, Espeset A, Mikati I, Bolduc I, Kulhanek R, White WA, Kenzie S, Snell-Rood EC. 2016 Nutrition shapes life-history evolution across species. *Proc. R. Soc. B* **283**, 20152764. (doi:10.1098/rspb.2015.2764)
25. Dunbar RIM, Shultz S. 2017 Why are there so many explanations for primate brain evolution? *Phil. Trans. R. Soc. B* **372**, 20160244. (doi:10.1098/rstb.2016.0244)
26. Slansky F, Scriber JM. 1985 Food consumption and utilization. In *Comprehensive insect physiology, biochemistry and pharmacology* (eds GA Kerkut, LI Gilbert), pp. 87–163. Oxford, UK: Pergamon Press.
27. Krenn HW. 2008 Feeding behaviours of neotropical butterflies (Lepidoptera, Papilionoidea). *Stapfia* (*Linz*) **88**, 295–304.
28. Boggs CL. 1981 Nutritional and life-history determinants of resource allocation in holometabolous insects. *Am. Nat.* **117**, 692–709. (doi:10.2307/2460754)
29. O'Brien DM, Boggs CL, Fogel ML. 2003 Pollen feeding in the butterfly *Heliconius charitonia*: isotopic evidence for essential amino acid transfer from pollen to eggs. *Proc. R. Soc. B* **270**, 2631–2636. (doi:10.1098/rspb.2003.2552)
30. Haydak MH. 1935 Brood rearing by honeybees confined to a pure carbohydrate diet. *J. Econ. Entomol.* **28**, 657–660. (doi:10.1093/jee/28.4.657)
31. Schmidt JO, Thoenes SC, Levin MD. 1987 Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources. *Ann. Entomol. Soc. Am.* **80**, 176–183. (doi:10.1093/aesa/80.2.176)
32. Martin CH, McGirr JA, Richards EJ, St. John ME. 2019 How to investigate the origins of novelty: insights gained from genetic, behavioral, and fitness perspectives. *Integr. Organismal Biol.* **1**, obz018. (doi:10.1093/iob/obz018)
33. Stroud JT, Losos JB. 2016 Ecological opportunity and adaptive radiation. *Annu. Rev. Ecol. Evol. Syst.* **47**, 507–532. (doi:10.1146/annurev-ecolsys-121415-032254)
34. Wilson GP, Evans AR, Corfe IJ, Smits PD, Fortelius M, Jernvall J. 2012 Adaptive radiation of multituberculate mammals before the extinction of dinosaurs. *Nature* **483**, 457–460. (doi:10.1038/nature10880)
35. Blount ZD, Borland CZ, Lenski RE. 2008 Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **105**, 7899–7906. (doi:10.1073/pnas.0803151105)
36. Meyer JR, Dobias DT, Weitz JS, Barrick JE, Quick RT, Lenski RE. 2012 Repeatability and contingency in the evolution of a key innovation in phage lambda. *Science* **335**, 428–432. (doi:10.1126/science.1214449)
37. Weber JN, Peterson BK, Hoekstra HE. 2013 Discrete genetic modules are responsible for complex burrow evolution in *Peromyscus* mice. *Nature* **493**, 402–405. (doi:10.1038/nature11816)
38. Hahn MW, Nakhleh L. 2016 Irrational exuberance for resolved species trees: Commentary. *Evolution* **70**, 7–17. (doi:10.1111/evo.12832)
39. DeVries PJ. 1979 Pollen-feeding rainforest *Parides* and *Battus* butterflies in Costa Rica. *Biotropica* **11**, 237–238. (doi:10.2307/2388045)
40. Krenn HW. 2010 Feeding mechanisms of adult Lepidoptera: structure, function, and evolution of the mouthparts. *Annu. Rev. Entomol.* **55**, 307–327. (doi:10.1146/annurev-ento-112408-085338)
41. Luo S, Li Y, Chen S, Zhang D, Renner SS. 2011 Gelechiidae moths are capable of chemically dissolving the pollen of their host plants: first documented sporopollenin breakdown by an animal. *PLoS ONE* **6**, e19219. (doi:10.1371/journal.pone.0019219)
42. Baker HG, Baker I. 1973 Amino-acids in nectar and their evolutionary significance. *Nature* **242**, 117–118. (doi:10.1038/227680a0)
43. Erhardt A, Baker I. 1990 Pollen amino acids - an additional diet for a nectar-feeding butterfly? *Plant Syst. Evol.* **169**, 111–121. (doi:10.1007/BF00935989)
44. Alm J, Ohnmeiss TE, Lanza J, Vriesenga L. 1990 Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia* **84**, 53–57. (doi:10.1007/BF00665594)
45. Mevi-Schütz J, Erhardt A. 2004 Mating frequency influences nectar amino acid preference of *Pieris napi*. *Proc. R. Soc. B* **271**, 153–158. (doi:10.1098/rspb.2003.2579)
46. Hill CJ, Pierce NE. 1989 The effect of adult diet on the biology of butterflies - 1. The common imperial blue, *Jalmenus evagoras*. *Oecologia* **81**, 249–257. (doi:10.1007/BF00379812)
47. Molleman F, Ding J, Wang J-L, Brakefield PM, Carey JR, Zwaan BJ. 2008 Amino acid sources in the adult diet do not affect life span and fecundity in the

fruit-feeding butterfly *Bicyclus anynana*. *Ecol. Entomol.* **33**, 429–438. (doi:10.1111/j.1365-2311.2008.00986.x)

48. O'Brien DM, Schrag DP, Martínez Del Rio C. 2000 Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. *Ecology* **81**, 2822–2831. (doi:10.1890/0012-9658(2000)081[2822:ATRIAH]2.0.CO;2)
49. Murphy DD, Launer AE, Ehrlich PR. 1983 The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. *Oecologia* **56**, 257–263. (doi:10.1007/BF00379699)
50. Mevi-Schütz J, Erhardt A. 2005 Amino acids in nectar enhance butterfly fecundity: a long-awaited link. *Am. Nat.* **165**, 411–419. (doi:10.1086/429150)
51. Boggs CL. 1997 Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. *Ecology* **78**, 192–202. (doi:10.1890/0012-9658(1997)078[0192:DORAFJ]2.0.CO;2)
52. Levin E, McCue MD, Davidowitz G. 2017 More than just sugar: allocation of nectar amino acids and fatty acids in a Lepidopteran. *Proc. R. Soc. B* **284**, 20162126. (doi:10.1098/rspb.2016.2126)
53. Cahenzli F, Erhardt A. 2013 Nectar amino acids enhance reproduction in male butterflies. *Oecologia* **171**, 197–205. (doi:10.1007/s00442-012-2395-8)
54. Beck J. 2007 The importance of amino acids in the adult diet of male tropical rainforest butterflies. *Oecologia* **151**, 741–747. (doi:10.1007/s00442-006-0613-y)
55. Molleman F, Zwaan B, Brakefield P, Carey J. 2007 Extraordinary long life spans in fruit-feeding butterflies can provide window on evolution of life span and aging. *Exp. Gerontol.* **42**, 472–482. (doi:10.1016/j.exger.2007.01.008)
56. Krenn HW, Penz CM. 1998 Mouthparts of *Heliconius* butterflies (Lepidoptera: Nymphalidae): a search for anatomical adaptation to pollen-feeding behavior. *Int. J. Insect Morphol. Embryol.* **27**, 301–309. (doi:10.1016/S0020-7322(98)00022-1)
57. Bauder J, Krenn HW. 2009 Muskelanordnung im Rüssel von pollenfressenden und nektrasaugenden Heliconiini. *Entomol. Austriaca* **16**, 159–160.
58. Penz CM, Krenn HW. 2000 Behavioral adaptations to pollen-feeding in *Heliconius* butterflies (Nymphalidae, Heliconiinae): an experiment using Lantana flowers. *J. Insect Behav.* **13**, 865–880.
59. Hikl A-L, Krenn HW. 2011 Pollen processing behavior of *Heliconius* butterflies: a derived grooming behavior. *J. Insect Sci.* **11**, 1–13. (doi:10.1673/031.011.9901)
60. Eberhard SH, Nemeschkal HL, Krenn HW. 2009 Biometrical evidence for adaptations of the salivary glands to pollen feeding in *Heliconius* butterflies (Lepidoptera: Nymphalidae). *Biol. J. Linn. Soc.* **97**, 604–612. (doi:10.1111/j.1095-8312.2009.01243.x)
61. Eberhard SH, Hrassnigg N, Crailsheim K, Krenn HW. 2007 Evidence of protease in the saliva of the butterfly *Heliconius melpomene* (L.) (Nymphalidae, Lepidoptera). *J. Insect Physiol.* **53**, 126–131. (doi:10.1016/j.jinsphys.2006.11.001)
62. Harpel D, Cullen DA, Ott SR, Jiggins CD, Walters JR. 2015 Pollen feeding proteomics: salivary proteins of the passion flower butterfly, *Heliconius melpomene*. *Insect Biochem. Mol. Biol.* **63**, 7–13. (doi:10.1016/j.ibmb.2015.04.004)
63. Smith G, Macias-Muñoz A, Briscoe AD. 2016 Gene duplication and gene expression changes play a role in the evolution of candidate pollen feeding genes in *Heliconius* butterflies. *Genome Biol. Evol.* **8**, 2581–2596. (doi:10.1093/gbe/evw180)
64. Kafatos F, Tartakoff A, Law J. 1967 Preliminary characterization of a proteolytic enzyme from silk moths. *J. Biol. Chem.* **242**, 1477–1487.
65. Mackenzie G, Boa AN, Diego-Taboada A, Atkin SL, Sathyapalan T. 2015 Sporopollenin, the least known yet toughest natural biopolymer. *Front. Mater.* **2**, 66. (doi:10.3389/fmats.2015.00066)
66. Krenn HW, Eberhard MJB, Eberhard SH, Hikl AL, Huber W, Gilbert LE. 2009 Mechanical damage to pollen aids nutrient acquisition in *Heliconius* butterflies (Nymphalidae). *Arthropod-Plant Interact.* **3**, 203–208. (doi:10.1007/s11829-009-9074-7)
67. Hassell MP, Southwood TRE. 1978 Foraging strategies of insects. *Annu. Rev. Ecol. Syst.* **9**, 75–98. (doi:10.1146/annurev.es.09.110178.000451)
68. Smid HM, Vet LEM. 2016 The complexity of learning, memory and neural processes in an evolutionary ecological context. *Curr. Opin. Insect Sci.* **15**, 61–69. (doi:10.1016/j.cois.2016.03.008)
69. Sivinski J. 1989 Mushroom body development in Nymphalid butterflies: a correlate of learning? *J. Insect. Behav.* **2**, 277–283. (doi:10.1007/BF01053299)
70. Montgomery SH, Merrill RM, Ott SR. 2016 Brain composition in *Heliconius* butterflies, posteclosion growth and experience-dependent neuropil plasticity. *J. Comp. Neurol.* **524**, 1747–1769. (doi:10.1002/cne.23993)
71. Murawski DA, Gilbert LE. 1986 Pollen flow in *Psiguria warscewiczii*: a comparison of *Heliconius* butterflies and hummingbirds. *Oecologia* **68**, 161–167. (doi:10.1007/BF00384782)
72. Condon MA, Gilbert LE. 1990 Reproductive biology and natural history of the neotropical vines *Gurania* and *Psiguria*. In *Biology and utilization of the cucurbitaceae* (eds DM Bates, RW Robinson, C Jeffrey), pp. 150–166. Ithaca, NY: Cornell University Press.
73. Zimmerman JK, Wright SJ, Calderón O, Pagan MA, Paton S. 2007 Flowering and fruiting phenologies of seasonal and aseasonal neotropical forests: the role of annual changes in irradiance. *J. Trop. Ecol.* **23**, 231–251. (doi:10.1017/S0266467406003890)
74. Heinrich B. 1979 Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia* **40**, 235–245. (doi:10.1007/BF00345321)
75. Janzen DH. 1971 Euglossine bees as long-distance pollinators of tropical plants. *Science* **171**, 203–205. (doi:10.1126/science.171.3967.203)
76. Benson WW. 1972 Natural selection for Müllerian mimicry in *Heliconius erato* in Costa Rica. *Science* **176**, 936–939. (doi:10.1126/science.176.4037.936)
77. Mallet J, Longino JT, Murawski D, Murawski A, Gamboa ASD, Journal T, Jun N. 1987 Handling effects in *Heliconius*: where do all the butterflies go? *J. Anim. Ecol.* **56**, 377–386.
78. Logan CJ *et al.* 2018 Beyond brain size: uncovering the neural correlates of behavioral and cognitive specialization. *Comp. Cogn. Behav. Rev.* **13**, 55–89. (doi:10.3819/CCBR.2018.130008)
79. Sherry DF, Vaccarino AL, Buckenham K, Herz RS. 1989 The hippocampal complex of food-storing birds. *Brain Behav. Evol.* **34**, 308–317. (doi:10.1159/000116516)
80. Farris SM. 2013 Evolution of complex higher brain centers and behaviors: behavioral correlates of mushroom body elaboration in insects. *Brain Behav. Evol.* **82**, 9–18. (doi:10.1159/000352057)
81. Mizunami M, Weibrecht JM, Strausfeld NJ. 1998 Mushroom bodies of the cockroach: their participation in place memory. *J. Comp. Neurol.* **402**, 520–537. (doi:10.1002/(SICI)1096-9861(19981228)402:4<520::AID-CNE6>3.0.CO;2-K)
82. Kamhi JF, Barron AB, Narendra A. 2020 Vertical lobes of the mushroom bodies are essential for view-based navigation in Australian *Myrmecia* ants. *Curr. Biol.* **30**, 1–6. (doi:10.1016/j.cub.2020.06.030)
83. Buehlmann C, Wozniak B, Goulard R, Webb B, Graham P, Niven JE. 2020 Mushroom bodies are required for learned visual navigation, but not for innate visual behavior, in ants. *Curr. Biol.* **30**, 3438–3443; e2. (doi:10.1016/j.cub.2020.07.013)
84. Farris SM, Schulmeister S. 2011 Parasitoidism, not sociality, is associated with the evolution of elaborate mushroom bodies in the brains of hymenopteran insects. *Proc. R. Soc. B* **278**, 940–951. (doi:10.1098/rspb.2010.2161)
85. van Nouhuys S, Kaartinen R. 2008 A parasitoid wasp uses landmarks while monitoring potential resources. *Proc. R. Soc. B* **275**, 377–385. (doi:10.1098/rspb.2007.1446)
86. Kühn-Bühlmann S, Wehner RW. 2006 Age-dependent and task-related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. *J. Neurobiol.* **66**, 511–521. (doi:10.1002/neu)
87. Pfeiffer K, Homberg U. 2014 Organization and functional roles of the central complex in the insect brain. *Annu. Rev. Entomol.* **59**, 165–184. (doi:10.1146/annurev-ento-011613-162031)
88. Varga AG, Kathman ND, Martin JP, Guo P, Ritzmann RE. 2017 Spatial navigation and the central complex: sensory acquisition, orientation, and motor control. *Front. Behav. Neurosci.* **11**, 4. (doi:10.3389/fnbeh.2017.00004)
89. de Castro ÉCP, Zagrobelny M, Cardoso MZ, Bak S. 2017 The arms race between heliconiine butterflies and *Passiflora* plants: new insights on an ancient subject. *Biol. Rev.* **93**, 555–573. (doi:10.1111/brv.12357)
90. Dell'Aglio DD, Losada ME, Jiggins CD. 2016 Butterfly learning and the diversification of plant leaf shape.

Front. Ecol. Evol. **4**, 81. (doi:10.3389/FEVO.2016.00081)

91. van Dijk LJA, Janz N, Schapers A, Gamberale-Stille G, Carlsson MA. 2017 Experience-dependent mushroom body plasticity in butterflies: consequences of search complexity and host range. *Proc. R. Soc. B* **284**, 20171594. (doi:10.1098/rspb.2017.1594)
92. Snell-Rood EC, Papaj DR, Gronenberg W. 2009 Brain size: a global or induced cost of learning? *Brain Behav. Evol.* **73**, 111–128. (doi:10.1159/000213647)
93. Nahrstedt A, Davis RH. 1985 Biosynthesis and quantitative relationships of the cyanogenic glucosides, linamarin and lotaustralin, in genera of the Heliconiini (Insecta: Lepidoptera). *Comp. Biochem. Phys. B* **82**, 745–749. (doi:10.1016/0305-0491(85)90519-X)
94. DeCasien AR, Thompson NA, Williams SA, Shattuck MR. 2018 Encephalization and longevity evolved in a correlated fashion in Euarchontoglires but not in other mammals. *Evolution* **72**, 2617–2631. (doi:10.1111/evo.13633)
95. Minias P, Podlaszczuk P. 2017 Longevity is associated with relative brain size in birds. *Ecol. Evol.* **7**, 3558–3566. (doi:10.1002/ece3.2961)
96. Chai P, Srygley RB. 1990 Predation and the flight, morphology, and temperature of neotropical rainforest butterflies. *Am. Nat.* **135**, 748–765. (doi:10.1086/285072)
97. Chai P. 1990 Relationships between visual characteristics of rainforest butterflies and responses of a specialized insectivorous bird. In *Adaptive coloration in invertebrates* (ed. M Wicksten), pp. 31–60. Galveston, TX: Texas A&M University.
98. Brower LP, Brower JVZ, Collins CT. 1963 Experimental studies of mimicry: relative palatability and Müllerian mimicry among neotropical butterflies of the subfamily Heliconiinae. *Zoolog. New York* **48**, 65–84.
99. de Castro ÉCP, Demirtas R, Orteu A, Olsen CE, Motawie MS, Cardoso MZ, Zagrobelny M, Bak S. 2020 The dynamics of cyanide defences in the life cycle of an aposematic butterfly: biosynthesis versus sequestration. *Insect Biochem. Mol. Biol.* **116**, 103259. (doi:10.1016/j.ibmb.2019.103259)
100. Cardoso MZ, Gilbert LE. 2013 Pollen feeding, resource allocation and the evolution of chemical defence in passion vine butterflies. *J. Evol. Biol.* **26**, 1254–1260. (doi:10.1111/jeb.12119)
101. de Castro ÉCP, Zagrobelny M, Zurano JP, Cardoso MZ, Feyereisen R, Bak S. 2019 Sequestration and biosynthesis of cyanogenic glucosides in passion vine butterflies and consequences for the diversification of their host plants. *Ecol. Evol.* **9**, 5079–5093. (doi:10.1002/ece3.5062)
102. Sculfort O, de Castro ÉCP, Kozak KM, Bak S, Elias M, Nay B, Llaurens V. 2020 Variation of chemical compounds in wild Heliconiini reveals ecological factors involved in the evolution of chemical defenses in mimetic butterflies. *Ecol. Evol.* **10**, 2677–2694. (doi:10.1002/ece3.6044)
103. Burger JMS, Kolss M, Pont J, Kawecki TJ. 2008 Learning ability and longevity: a symmetrical evolutionary trade-off in *Drosophila*. *Evolution* **62**, 1294–1304. (doi:10.1111/j.1558-5646.2008.00376.x)
104. Snell-Rood EC, Davidowitz G, Papaj DR. 2011 Reproductive tradeoffs of learning in a butterfly. *Behav. Ecol.* **22**, 291–302. (doi:10.1093/beheco/arq169)
105. Burns JG, Foucaud J, Mery F. 2011 Costs of memory: lessons from 'mini' brains. *Proc. R. Soc. B* **278**, 923–929. (doi:10.1098/rspb.2010.2488)
106. Niven JE, Laughlin SB. 2008 Energy limitation as a selective pressure on the evolution of sensory systems. *J. Exp. Biol.* **211**, 1792–1804. (doi:10.1242/jeb.017574)
107. Pyke GH. 2010 Optimal foraging theory: introduction. In *Encyclopedia of animal behavior* (eds MD Breed, J Moore), pp. 601–603. Burlington, MA: Elsevier.
108. Grimm V, Railsback S. 2005 *Individual-based modeling and ecology*. Princeton, NJ: Princeton University Press.
109. Montgomery SH, Mundy NI, Barton RA. 2016 Brain evolution and development: adaptation, allometry and constraint. *Proc. R. Soc. B* **283**, 20160433. (doi:10.1098/rspb.2016.0433)
110. Uyeda JC, Zenil-Ferguson R, Pennell MW. 2018 Rethinking phylogenetic comparative methods. *Syst. Biol.* **67**, 1091–1109. (doi:10.1093/sysbio/syy031)
111. Nussey DH, Froy H, Lemaitre J-F, Gaillard J-M, Austad SN. 2013 Senescence in natural populations of animals: widespread evidence and its implications for bio-gerontology. *Ageing Res. Rev.* **12**, 214–225. (doi:10.1016/j.arr.2012.07.004)
112. Brown S, Strausfeld NJ. 2009 The effect of age on a visual learning task in the American cockroach. *Learn. Mem.* **16**, 210–223. (doi:10.1101/lm.1241909)
113. Tofilski A. 2000 Senescence and learning in honeybee (*Apis mellifera*) workers. *Acta Neurobiol. Exp.* **60**, 35–39.